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RESUMEN

LA PRODUCCION DE LECHE CON UNA ALIMENTACION CARENTE DE PROTEINA, USANDO UREA Y SALES AMONICAS COMO UNICA FUENTE DE NITROGENO

Los experimentos que se han realizado durante muchos años, han demostrado que se puede obtener una considerable producción de leche con vacas sometidas a una alimentación carente de proteína. Esto se ha demostrado decisivamente con experimentos de alimentación en los que se ha utilizado almidón purificado, sacarosa y α-celulosa con urea y una pequeña cantidad de sales de amonio como única fuente de nitrógeno. Utilizando 650 gr. de urea al día durante el mejor período productivo, la producción anual de leche se ha elevado a más de 4.000 kg. de leche standard (684 Kcal/Kg. leche). Las investigaciones han demostrado que la composición de la proteína de la leche es similar en la alimentación experimental carente de proteína a la de la alimentación normal. El contenido de las vitaminas hidrosolubles de la leche alcanza también el mismo nivel. La composición de la grasa de la leche depende, sin embargo, de la naturaleza y la calidad de la grasa incluida en la alimentación. El sabor de la leche es muy similar al de la leche producida con alimentación normal.

Las investigaciones sobre la alimentación carente de proteínas han abierto nuevas posibilidades a la producción de leche. Como las proteínas pueden ser sustituidas por urea, que la industria puede producir a gran escala económicamente en una cantidad casi ilimitada, los forrajes pobres en proteínas —cuya utilización ha sido prácticamente imposible hasta ahora debido a su falta de proteína— pueden constituir la ración de las vacas lecheras. Los ensayos de naturaleza práctica sobre alimentación que se están realizando en la actualidad, han demostrado que en países ricos en bosques, una parte considerable del alimento, se puede preparar a partir de madera. La fracción hemicelulósica se puede solubilizar con vapor a presión elevada. La caña de azúcar es el forraje de hidratos de carbono más barato en aquellas regiones donde se cultiva. Las raciones preparadas de diferentes fuentes de hidratos de carbono se pueden usar en diversas partes del globo.

In milk production the daily feed has to contain roughly 60 g digestible crude protein for each kilogram of milk produced, in addition to the 300 g necessary for the maintenance of the cow That is nearly twice as much protein as there is in one kilogram of milk. Because the utilization of the nutrients in the organism of ruminants is very different from that of other mammals, the fermentation processes in the rumen caused by microbial flora being of decisive importance, a part of the protein in their feed can consist of simple nitrogen compounds e. g. amides or ammonia. This view was presented already in 1891 by Zuntz in Germany, but still the utilization of non-protein nitrogen in the rumen is not well understood (*). A great number of feeding experiments have been made in different countries to find out how much of the protein can be successfully substituted by urea, which is readily decomposed to ammonia in the rumen. The problem is complicated when the question is of normal feeding with plenty of protein.

In experiments in which the purified feed used does not contain protein, and in which urea is used as the only significant source of nitrogen, the interpretation of the results is much clearer. These kinds of experiments have been made especially in the United States with growing lambs, goats and steers. The ruminal biosynthesis of all protein amino acids, even of essential amino acids was demonstrated in these experiments (Loosli *et al.*, Duncan *et al.*, Tillman *et al.*). In spite of the relatively small amounts of protein which are needed for the growth of young ruminants — e. g. for the rearing of a heifer about 300-350 g of digestible crude protein per day — no optimum growth was obtained with urea as the sole source of nitrogen. Woods and Tillman in 1956 compared the effect of salt mixtures of different acidity on the growth of sheep in experiments with purified diet and urea as the sole source of nitrogen. Also with an alcaline mixture, which is the natural one, the growth of sheep was inferior when urea was used to that obtained with soya protein. As is revealed from the foregoing, a cow producing for instance 15 kg milk per day has to receive about 1200 g digestible crude protein. It is thus understandable that milk production on a protein-free feed had not been investigated up till our experiments were started.

However, many problems, e. g. the capacity of the microbial flora of the rumen for synthesis of amino acids and proteins, the origin of milk flavor, and the biosynthesis of different milk components, actually require the study of milk production on a purified feed, with urea and ammonium salts as the sole source of nitrogen. Such a study is of course fundamental also from the practical point of view.

(*) References: See the article of the author in *Science*, vol. 153, n.º 3744, pp. 1603-1614, 1966.

Our studies on the production of milk with a purified, protein-free feed, using mostly urea and small amounts of ammonium salts as the sole sources of nitrogen, were started in connection with a research project concerning the origin of the flavor substances of milk. The question under study was to what extent the flavor substances are due to synthesis within the cow's organism and to what extent they are derived from the feed. It was thus important to prepare a test feed which was as simple and as purified as possible. Proteins were replaced by the simple nitrogen compounds urea and ammonium salts, and the complex carbohydrates and other organic compounds present in plants by purified starch, cellulose and sucrose.

Feeding experiments with $^{15}NH^{+}_{4}$ and ^{15}N-urea

In our experiments performed in the spring of 1958, in which a cow on normal feeding was given as one dose ammonium sulphate labelled with ^{15}N, the labelling of the amino acids separated by fractionation after the hydrolysis of the milk proteins was determined. It was shown that in the milk obtained 15 hours after the feeding of the ^{15}N-ammonium sulphate all the protein amino acids studied were labelled, but that the degree of labelling of the various amino acids was different. The essential amino acids were labelled more weakly than the non-essential, which was natural since the former are obviously formed only in the rumen by microorganisms, the latter further in animal organism, especially in the liver, from ammonia. Taking the labelling of glutamic acid, which was labelled more rapidly than any other amino acid, as 100, the labelling of valine was 59, lysine 54, phenylalanine 50, arginine 41, but histidine only 15.

On the basis of this experiment, it was confirmed that the microbial flora of the rumen is able to synthesize all the protein amino acids and demonstrated that the amino acids formed were used for the synthesis of milk protein. The weak labelling of some amino acids suggested that their synthesis may form a bottleneck in protein synthesis. Thus the possibility of developing in the rumen by adaptation a microbial flora which could synthesize protein from ammonia more effectively than the rumen microorganisms of cows on normal feeding and thus make the milk production possible seemed to deserve experimental study.

The experiments on protein-free feed with urea-and ammonium-N as the sole source of nitrogen were started with one cow in the autumn 1961 and with another at the beginning of 1962 using a very slow adaptation. When one of the test cows (Eiru) was given a dose of ^{15}N-urea after the cow had been on the test feeding six months and the labelling of the amino acids of milk protein was estimated 6.3 and

20 h after the administration of ^{15}N, the labelling of essential amino acids generally and especially of histidine and tryptophan was strongly increased when compared with the values obtained in experiments with a non-adapted cow. Another experiment in which ^{15}N-ammonium sulphate was given the same test cow after it had been on test feed 25 months, gave similar results. The results show that a dose of both ^{15}N-urea and ^{15}N-ammonium sulphate has labelled most of the amino acids of milk protein practically to the same degree and further that the test feed lasting for 6 and 25 months has had a similar effect on labelling. The curves in Fig. 1 illustrate the effect of the adaptation on the labelling of some essential amino acids of milk protein.

TEST FEED AND MILK PRODUCTION

The test feed has been made up of the following components (Table 1). Most of the time the starch used has been potato starch containing only 0.02 % N, whereas the maize starch contains 0.09 % N. The amount of nitrogen-containing impurities in the test feed is so small that the urea and ammonium nitrogen account for 99.5 % of the total nitrogen.

Different test cows were given briquettes, cellulose-rich paste and cellulose strips in different proportions according to appetite. The feeding of one cow differed thus from the feeding of the others to some extent. Roughly speaking it can be said that the test feed comprised potato starch 50-55 %, cellulose 25-30 % and sucrose 17-23 % of the total carbohydrates. The success of the urea feeding is decisively dependent on the composition of the carbohydrates fed. The portion of starch in the feed can hardly be reduced very much without a drop in milk production.

The adaptation of the cows to the test feed was generally arranged so that the amount of normal fodder was gradually decreased and the amount of test feed was correspondingly increased. The transfer to the test feed with the first two cows was made during a period of 4 6 months; later when new cows were adapted this was 2 or 3 months. The test cows were fed twice a day. The cows on higher production (more than 10-12 kg milk/day) ate their larger rations gradually all the day and regulated in this way the intake of urea. There was thus no need to divide the feed repeatedly into small portions. In contrast, the dry cows consumed their small rations in a very short time.

As appears from Table 1 the nitrogen content of the feed has been raised considerably during the experiment. For fear of ammonia poisoning urea and ammonium salts were at first used cautiously. The largest amount of urea (ammonium nitrogen included) during the first

TABLE 1

Composition of the components of the test feed

1. The composition of briquettes, about 9 g each, was:

	1962-64	1965
α-Cellulose powder	9.5 %	9.9 %
Starch	57.0 »	52.9 »
Sucrose	20.9 »	23.1 »
Salt mixture	8.2 »	8.9 »
Urea (94 %) + ammonium salts (6 %) (calc. as urea)	4.4 »	5.2 »
Total	100.0 %	
Water content	15.0 »	

2. The composition of the wet cellulose paste was:

	1962-63	1965
α-Cellulose powder	60.3 %	57.3 %
Starch	19.5 »	16.4 »
Sucrose	12.1 »	12.2 »
Salt mixture	7.1 »	8.8 »
Urea	1.0 »	5.3 »
Total	100.0 %	
Mixed with water. Water content	75.0 »	

3. Cellulose strips with precipitation of silicic acid or without it, 75 % water, urea 4.0 % + salt mixture 3.0 %/dry substance.

4 Plant oils, 50-130 g/cow/day.

5. Vitamins: A (37500-100000 IU/cow/day) and $D_2 + D_3$ (7500-20000 IU/cow/day.

6. Vitamin E (α-tocopherol) 30 mg/cow/day in 1965, after that 330-500 mg/cow/day; during the first years no vitamin E preparation was used. The composition of the salt mixture has been changed to some extent during the experiment on the basis of the analyses of faeces. The mixture contains at present (g/100 g dry mixture): Na 6.9, K 12.5, Ca 14.4, Mg 5.3, Cl 11.0, S (as sulphate) 3.8, P 14.8, Fe 0.238, Zn 0.085, Mn 0.038, Cu 0.015, Se 0.003, B 0.003, Co 0.001, I 0.001, Mo 0.0006.

two years was only a little more than 400 g per day during the highest milk production. As investigations on the rumen contents and blood showed, however, that ammonia had not accumulated in the rumen and that the ammonia content of the blood had not increased, the amount of nitrogen in the feed was raised to such an extent that since autumn of 1965 the cows, weighing about 450 kg, could receive as much as 600 g urea per day.

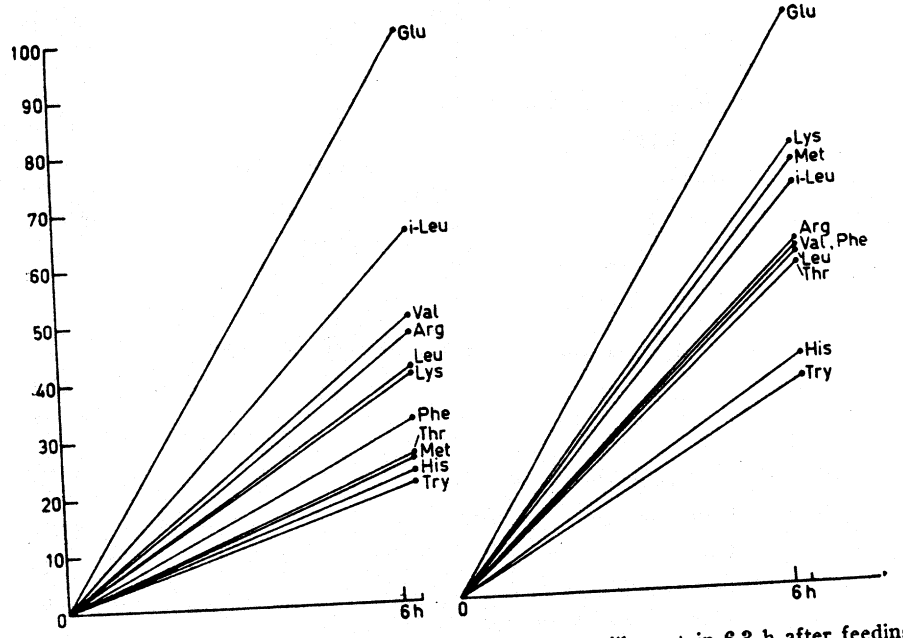
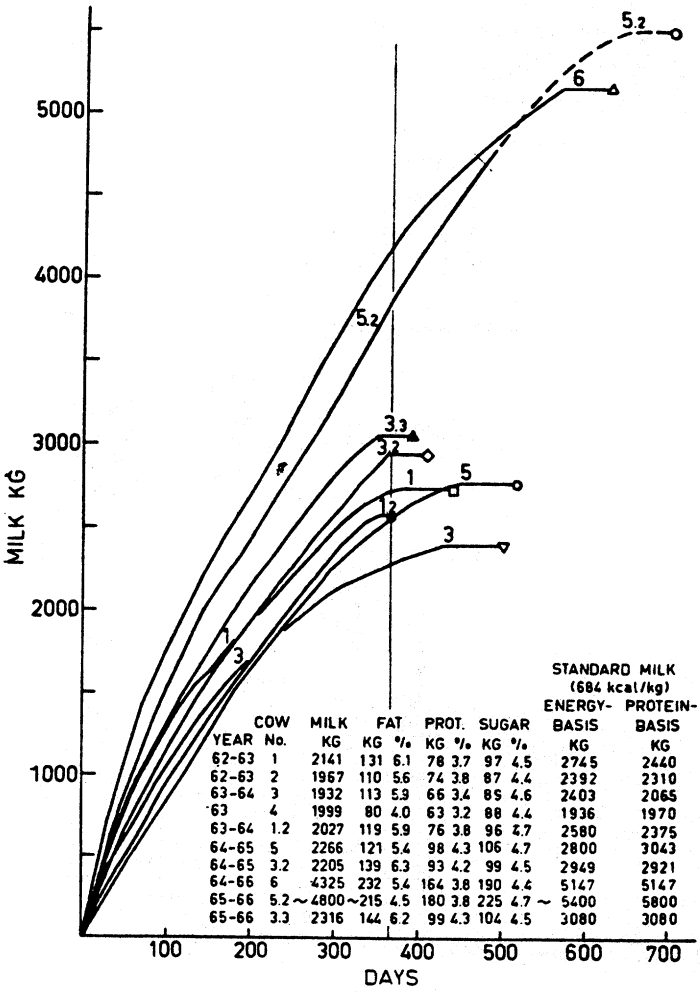


Fig. 1.—Labelling of the essential amino acids of total milk protein 6.3 h after feeding a single dose of ^{15}N-urea. The results are expressed as a percentage of the labelling of glutamic acid. On the left a feeding experiment with a cow on normal feeding (17.3.1966), on the right a feeding experiment with a test cow (20.10.1962) 6 months after the start of the experimental feeding. Histidine and tryptophan have the lowest labelling in both experiments but the increase in their labelling on the experimental feed is remarkable. (Estimations by M. Kreula and T. Moisio.)

The digestibility of the urea and the nitrogen balance was estimated several times using both the method of collecting faeces and urine separately and the method of feeding chromic oxide (Cr_2O_3) as the marker and collecting faeces and urine together. In addition when the digestibility of nitrogen alone was to be estimated the chromic oxide method was employed. The raising of urea in the feed enhanced the digestion coefficient of urea-N from 63 ± 0.97 to 70 ± 1.2 when the nitrogen consumed (g N/kg org. feed) was raised from about 18 to 23, respectively. The increase in the coefficients when more urea was fed

was statistically highly significant. The nitrogen balance (g/day) averaged about + 13 on higher urea ration.

The raising of urea increased the milk yield very much. It also greatly reduced or totally removed a symptom of probable nitrogen deficiency



YEAR	COW No.	MILK KG	FAT KG	FAT %	PROT. KG	PROT. %	SUGAR KG	SUGAR %	STANDARD MILK (684 kcal/kg) ENERGY-BASIS KG	STANDARD MILK (684 kcal/kg) PROTEIN-BASIS KG
62-63	1	2141	131	6.1	78	3.7	97	4.5	2745	2440
62-63	2	1967	110	5.6	74	3.8	87	4.4	2392	2310
63-64	3	1932	113	5.9	66	3.4	85	4.6	2403	2065
63	4	1999	80	4.0	63	3.2	88	4.4	1936	1970
63-64	1.2	2027	119	5.9	76	3.8	96	4.7	2580	2375
64-65	5	2266	121	5.4	98	4.3	106	4.7	2800	3043
64-65	3.2	2205	139	6.3	93	4.2	99	4.5	2949	2921
64-66	6	4325	232	5.4	164	3.8	190	4.4	5147	5147
65-66	5.2	~4800	~215	4.5	180	3.8	225	4.7	~ 5400	5800
65-66	3.3	2316	144	6.2	99	4.3	104	4.5	3080	3080

Fig. 2.—Milk production of the test cows on experimental feeding (see the text in the figure). The milk yield calculated both on the energy basis (standard milk = 684 Kcal/kg milk) and protein basis (3.2 % protein). The numbers of the curves refer to the test cows, the small numbers the calving times during the experimental feeding (e. g. 3 first calving, 3.2 second, 3.3 third calving). the end points of the curves the calving days. The annual milk yields can be seen from the curves. The feeding experiments were performed in cooperation with M. Lampila (1961-63) and T. Ettala (1963-).

which was observed when the lower nitrogen feed was used: the continuous thinning or loss of the hairy coat of the fore part of the legs of the cows, especially that of the hind legs about two months after calving and the rapid regrowth when the daily milk yield had decreased to about 7 kg.

The oestrus of the test cows has been regular and easy to observe. However, many of them have required several services. Each of the test cows has become pregnant while two of the test cows, Eiru and Aino have already calved three times. It is possible that at least one of the

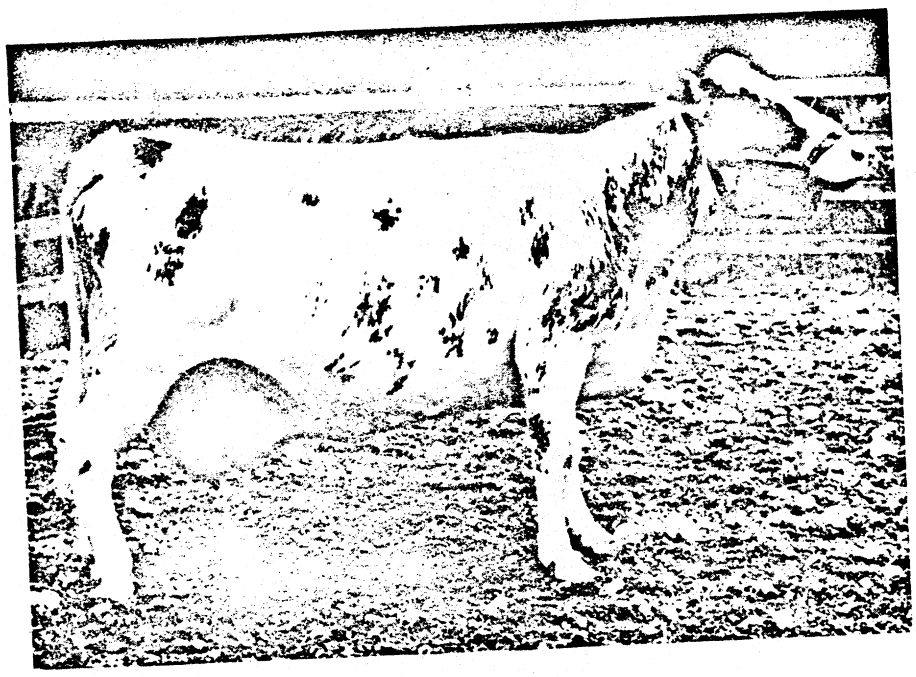


Fig. 3.—Test cow Metta after being on test feed 370 days from calving.

reasons for the requirement of several services is the deficiency of vitamin E. The test cows were not given vitamin E preparations up till the end of 1964. During 1965 they were fed daily 30 mg α-tocopherol/cow and since January 1966 330-500 mg (Table 1). The vitamin E effect of the plant oil fed is probably not sufficient to satisfy the need of the milking cow for this vitamin especially because polyunsaturated fatty acids enhance the requirement of vitamin E.

The production of the test cows on the experimental feeding during the different lactation periods can be seen in Fig. 2, which shows the yield of milk, milk fat, milk protein and milk sugar of each cow. In the figure also the milk yields/year and the length of lactation periods

are seen. In the column next to the last the milk yield has been calculated on an energy basis (standard milk = 684 Kcal/kg milk) and in the last column on the basis of the protein content (basis 3.2 % protein). Values of 9 Kcal for fat and 4 Kcal for protein and sugar per gram have been used in the calculations.

TABLE 2

The nutritive value of the test feed expressed in Scand. feed units (fu) and the amount of nitrogen fed per feed unit

Jairu. calved second time on May 19, 65.
Calculations made for a period of 77 days, July 1. 65 — Sept. 15. 65
Weight on June 30, 65; 445 kg and on August 31. 65; 445 kg.

Milk production: 864 kg (5.1 % fat, 3.8 % protein, 4.8 % sugar) = 1010 kg standard milk = 13.1 kg/day.

Requirement of feed units for:

Maintenance	77 × 3.5 fu =	270 fu
Production	1010 × 0.37" fu =	374 fu
Total		644 fu

Fed: 660 kg carbohydrates (52.2 % starch. 25.2 % α-cellulose, 22.6 % sugar).

10 kg fat = 16.8 fu

670 kg org. subst.

1.05 kg carbohydrates = 1 fu.

Urea- and ammonium-N fed 16.8 kg (99.6 % of tot. N in feed), corresponding to 11.8 kg dig. N (digestibility 70 %) or *18.3 g dig. N/fu = 114 g dig. prot./fu.*

Fed: 25.1 g N/kg org. substance.

The curves in Fig. 2 show how greatly the milk yield has risen since 1964 after the increase of the amount of urea in the feed (s. Table 1). The highest production of standard milk per year has so far been 4217 kg and during a prolonged lactation period 5147 kg (No. 6, Metta).

The following terms for milk are used in this article: *Normal* milk = milk produced on normal feed (pasture, hay, silage, roots, concentrates, etc.), independent of the composition of the milk. *Zero milk*

(0-milk) = milk produced on a protein-free test feed, urea and ammonium salts as the sole source of nitrogen, *Standard milk* = milk containing 684 Kcal/kg milk, e. g. 4.0 % fat, 3.2 % protein, 4.9 % sugar. Milk

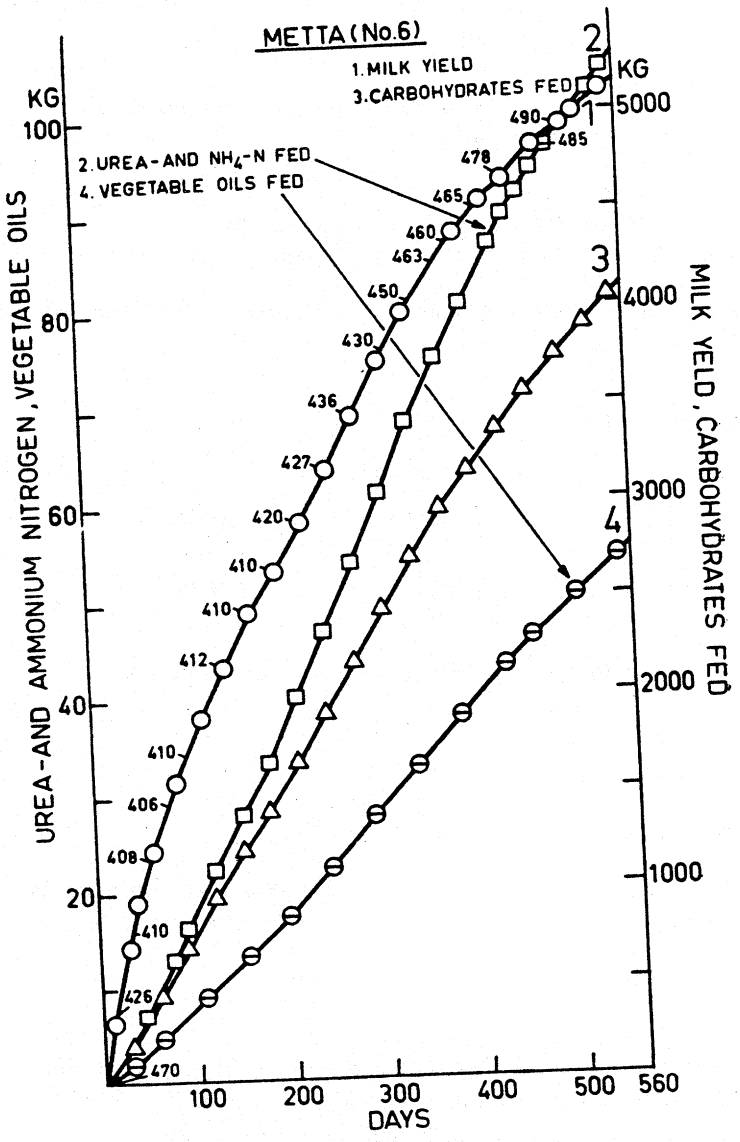


Fig. 4.—The production of milk and the intake of carbohydrates, urea-N (NH_4-N included) and vegetable oils of the test cow Metta.

of other composition is calculated to standard milk on an energy basis. Where the calculation has been made on a protein basis, milk with a protein content of 3.2 % is used as the basis.

The food value of the carbohydrates used was estimated by calculating the feed units on the basis of the amounts of feed consumed and the amount of milk produced. The calculations were made on data accumulated over a selected long period when the weight of the cow remained practically unchanged, so that weight increase or decrease could be omitted from the calculations. The calculations on the basis of the feed consumption and milk yield of test cow Jairu are presented in Table 2.

On the basis of similar calculations of three test cows, 1.05 to 1.13 kg carbohydrates correspond to one feed unit. Cellulose reduced the digestibility of the total carbohydrates because the cellulose powder in the briquettes and in the cellulose-rich paste may pass too quickly through the rumen to be digested. The faeces of the test cows actually contain mainly fine graded cellulose. In addition vigorously bleached sulphite cellulose has generally a lower digestibility than unbleached (Saarinen *et al.*). For α-cellulose powder used we found digestion coefficient of about 40-60.

The milk production and the intake of carbohydrates, urea-N (ammonium-N included) and vegetable oils of the test cow Metta are given in Fig. 4.

Microbial flora and protein synthesis in the rumen

The observations on the rapid utilization of ammonia in the rumen of the cows adapted to the test feed could be explained only by supposing that the microbial flora of the rumen content had been effectively adapted to the utilization of ammonium nitrogen. The investigations in this laboratory concerning the microbial flora of the rumen have shown that the number of protozoa in the rumen of the adapted cows has decreased enormously or that they are entirely absent, while the number of bacteria has risen remarkably, 50 × and more.

Only some preliminary studies of the bacterial flora of the rumen contents of the test cows have so far been made in this laboratory. Bacteria growing solely on ammonium nitrogen without specific factors are the most important for the test feed. In addition bacteria having higher requirements for their feed are probably necessary for the effective function of the microbial flora of the rumen. In his fundamental work Nurmikko studied the symbiotic growth of lactic acid bacteria and showed that different strains of the lactic acid bacteria can feed one another with different growth factors when grown in the same medium, because the factors synthesized in the bacterial cells are partly excreted into the medium. That means that in mixed cultures different strains can grow in a simpler medium than in pure cultures. The symbiotic growth is probably general in microbial associations and the

microbial flora of the rumen contents one of the most complicated examples of this type of system.

In the amino acid composition of the hydrolysate of the total protein of the rumen contents of test cows and control cows on normal feed systematic differences could be found only regarding α-ε-diamino-pimelic acid, a characteristic constituent of the cell wall of many bacteria (Work). It was regularly present in much higher amounts in the hydrolysate of the rumen protein of test cows (1.5 ± 0.1 % of total amino acids) thant that of normally-fed cows (0.4 ± 0.1 %).

Studies on the composition of the nitrogenous compounds of the rumen contents have shown that the ammonia concentration of cows on normal feed (protein-rich good silage, crushed oat and hay) is higher (11.1-26.6 mg/100 ml, av. 15.7 ± 1.5) than of test cows (0.7-10.6 mg/100 ml, av. 4.8 ± 1.3). The estimations were made 1 h after the samples of rumen contents were taken.

Nitrogenous compounds in faeces

The composition of the nitrogenous compounds of the faeces of the test cows has been closely studied in this laboratory. In experiments with rats, McNaught *et al.* found the digestion coefficient of the protein in a bacterial fraction grown in strained rumen liquor with urea and carbohydrate supplement to be 74. If this coefficient is the same with ruminants, the main part of the nitrogen of the faeces of the test cows should be indigestible bacterial protein. In fact among the normal amino acids which are found in the hydrolysate of the protein fraction of the faeces of the test cows α, ε-diamino-pimelic acid is present in much higher amounts than in the same fraction from cows on normal (av. 2.7 ± 0.49 and 0.6 ± 0.23 % of total amino acids of the hydrolysate, respectively). This is in accordance with the findings of the amino acid composition of the proteins of the rumen contents. Although all rumen bacteria do not contain diamino-pimelic acid in their cell wall «proteins» (Synge, Purser and Burchler) the high content of this amino acid of the protein fraction of faeces strongly suports other results presented above of the greatly enchanced bacterial protein synthesis in the rumen of the test cows.

Composition of the nitrogenous compounds of the blood of the test cows

Because the mammary gland receives the raw material for the synthesis of different components of milk from the blood the knowledge of the composition of the blood of the test cows is important. A great

number of analyses have been made of the whole blood and plasma of cows on test feed and for comparison also on normal feed. The blood samples, about 150 ml each, were taken from the jugular vein.

The free amino acids of the blood form only a very small part of the nitrogenous compounds of the blood, but they are decisively important for the formation of the proteins of milk in the mammary gland. On the basis of the latest studies the proteins of milk are mainly formed from the free amino acids found in the blood. Therefore in our laboratory attention has especially been directed towards the amounts of free amino acids in the blood plasma. The levels of most of the free amino acids, particularly the essential amino acids, in the plasma of the lactating test cows were lower than those of the control cows on normal feed. The relative decrease in the level of free histidine in plasma was on the test feed greater than that of any other amino acid. The histidine content decreased some weeks after calving, remained low for several months and rose again to some extent while the milk production decreased. When at its lowest level, the free histidine content of the plasma was about 20 % of the corresponding values of the normally-fed cows. The level of histidine and many other amino acids of dry test cows and heifers is much higher than of lactating cows (Fig. 5). The concentrations of plasma urea and total nitrogen were similar with the test and the control cows.

The total nitrogen of the blood of the milk-giving test cows is lower than that of normally-fed cows, due mainly to differences in the hemoglobin content. When the test cows are dry the hemoglobin content of their blood is normal (13-14 g/100 ml blood) but some weeks after calving the hemoglobin content decreases. In late lactation when the milk production is low the hemoglobin content rises gradually and reaches a maximum when the cows are dry. For instance the blood hemoglobin of the test cow Jairu when it was dry was 13.8 g/100 ml blood, one month after calving 12.9 g, three months 10.4 g, five months 10.3 g and eleven months 13.7 g (milk production still 8 kg/day). It is still not known what the reason is for the reduction of the hemoglobin level of a milk-giving cow on test feed. Since the amount of free histidine in the blood of the test cows is very low, and since on the basis of the observations presented above histidine seems to form a bottle-neck in protein synthesis with cows on the test feed, it may be that the primary reason is the deficiency of histidine during the post-calving period when the protein requirement is greatly increased because of milk production. As is known the histidine content of hemoglobin is high, more than 8 %. Also other factors can be suspected, e. g. the possible deficiency of vitamin B_{12}. There is no evidence of this hypothesis. The concentrations of most of the free amino acids and the unknown compounds in the whole blood were higher than in the plasma.

The amino acid composition of the whole blood protein of the test cows was generally similar to that of the normally-fed control cows. In electrophoretic studies on the serum proteins from several blood samples of the test and control cows carried out in this laboratory, only slight differences in the various protein fractions in samples from individual cows and between those of the test and control cows could be observed.

With the milk-giving test cows, the total cholesterol of blood plasma has averaged 69 ± 3 mg/100 ml plasma, and with two cows on normal feed 210 ± 23 mk/100 ml plasma. Esterified cholesterol accounted for an average of 87 % of the total plasma cholesterol both of the test and

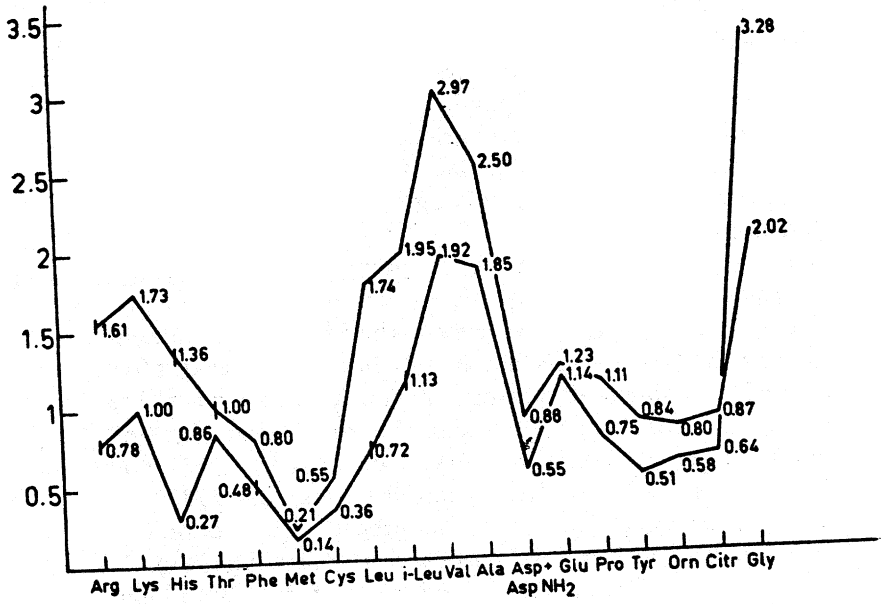


Fig. 5.—The contents of some free amino acids in the blood plasma of the lactating test cows (values on the lower curve, except glycine), and of normally-fed cows (values on higher curves, except glycine). The numbers signify free amino acids mg/100 ml plasma. The fall of the free histidine to 20 % of normal values is noteworthy.

normally-fed cows. The total plasma lipids of the test and normally-fed cows were 158 ± 8 and 522 ± 64/100 ml plasma, respectively. Total volatile fatty acids in the plasma of the test and normally-fed cows, in contrast, are almost on the same level.

Composition of the 0-milk

The composition of the 0-milk, when the quantitatively most important compounds are taken into consideration, corresponds to that of normal fat-rich milk as far as fat and protein are concerned. When

the content of urea in the feed was raised the fat content remained about the same or decreased somewhat, but the protein content rose remarkably. It thus seems that the amount of urea fed has a positive influence on the protein content of the 0-milk but not on the fat content. The sugar content of the 0-milk (4.4-4.7 %) has been somewhat lower than that of normal milk.

The composition of the nitrogenous substances, especially the proteins of the 0-milk, has received our special attention. At the beginning it was found that the ratio of the total N to residual N (N remaining after acid precipitation of casein) in 0-milk was very similar to that in normal milk. A great number of analyses have shown that the non-protein N is on the same level in the 0-milk and normal milk. The portion of NH_4-N + urea of total N has been even slightly lowover in 0-milk than in normal milk.

Amino acid composition of casein and total protein has been estimated after acid hydrolysis in hundreds of milk samples, though tryptophan has been determined in a few samples only. The values of the amino acids of the total protein and casein of the 0-milk are so close to those of milk produced on normal feed that conclusive differences could not be demonstrated.

Neither has the fractionation of the proteins shown any differences between the 0-milk and normal milk. Both the fractionation of the proteins of milk directly on a DEAE column and the fractionation of casein and the serum proteins of milk using gel-electrophoresis have shown the similarity between the 0-milk and normal milk. Small differences in some protein fractions of the milk of different cows can be observed, but they seem to be of an individual character. No conclusive differences in the activities of the milk enzymes studied could be found between 0-milk and normal milk when the appreciable individual variations are considered. The peroxidase, xanthinoxidase, aldolase, amylase, alkaline phosphatase and lipase activities have so far been estimated.

These results show how extraordinarily effective is the protein synthesis in the mammary gland. In spite of the low concentration of some free essential amino acids in the blood of the test cows the mammary gland is capable of synthesizing a surprising amount of milk protein, the numerous components of which have a normal composition. The high protein content and low urea and ammonium content of 0-milk is particularly remarkable.

When the 0-milk and normal milk are compared, the only macro constituent showing major differences in composition is the fat. As is well known, the composition of the milk fat is greatly influenced by the nature and amount of the fatty acids in the feed of the cow. Since there is practically no fat in our test feed apart from the vegetable oils added,

it has been possible to make significant observations on the biosynthesis of the fatty acids in our feeding experiments. In Table 3 some results of the fatty acid analyses of the 0-milk fat are seen.

The results show that 1) the palmitic acid content of the fat of the 0-milk was exceptionally high, almost 50 % of the total fatty acids, when the daily amount of oil fed was as low as 37 g per cow, the total production of fat of the test cow Eiru being 428 g and that of Jairu 358 g per day. When the amount of oil fed was doubled, the palmitic acid content decreased to about 40 %. Raising the amount of oil to 129 g decreased further the proportion of palmitic acid but it was still high. 2) The amount of oleic acid was correspondingly very low, about 10 %, when the smallest amount of oil was used in the feeding. The level was doubled when the greatest amount of oil was used, being still considerably lower than that of the milk fat produced on normal feed. The amount of stearic acid was especially low, rising only a little after the amount of oil in the feeding was increased. 3) The level of lower unsaturated fatty acids, especially C_{16}-and C_{14}-acids in the fat of the 0-milk was higher than in the fat of normal milk. 4) The amount of branched-chain fatty acids was also somewhat increased in the fat of the 0-milk.

On the basis of the production of milk fat and the composition of its fatty acids the hypothesis seems justified that the fatty acids of the milk fat of the test cows on low fat rations are synthesized mainly in the mammary gland and that the synthesis of the fatty acids containing more than 16 carbon atoms is weak. According to Riis there is evidence that with normal feed the synthesis within the mammary gland accounts for about one half of the fat in cow's milk, the other half being derived directly from the blood. The low content of C_{18}-acids in the fat of 0-milk together with the suppressed lipid content of the blood of the test cows suggest that a vigorous synthesis of fatty acids and fat occurs in the mammary gland and that the derivation from the blood is low.

The level of cholesterol in the 0-milk has been measured over a period of two years, with samples from herds on pasture or normal winter feed being taken as control (Homer and Virtanen). The mean value of 93 samples of normal milk was 317 $\pm$ 4 mg total cholesterol/100 g milk fat, whereas the 0-milk values were clearly higher, with a mean of 409 $\pm$ 6 mg. Esterified cholesterol was about 6 % of the total cholesterol in both 0-milk and control milk. Patton and McCarthy found evidence the cholesterol, in its intermediate esterified form, probably plays an important role in milk fat synthesis in the mammary gland. Considering this finding, and also that the concentration of lipid in the blood of the test cows is low, the higher cholesterol values of 0-milk may indicate that the proportion of the total milk fat synthesized within the mammary gland of the test cow is higher than the proportion with normal feed.

TABLE 3

The fatty acid composition of the milk fat on test feed and normal feed (E. Piironen)

Date of calving	Eiru December 11, 1963			Jairu Dec. 24, 63	Jairu May 19, 65	Normal cattle
Feeding period:						
Began	Jan. 1, 64	Jan. 29, 64	April 1, 64	Jan. 11, 64	July 11, 65	Sep. 25, 63
Ended	Jan. 28, 64	March 31, 64	June 30, 64	Feb. 20, 64	Sep. 20, 65	May 15, 64
Lasted days	28	63	91	41	72	23
Fat of diet g/day:						
Olive oil..	0.0	36.6	36.6	0.0	36.6	
Maize oil..	18.4	18.4	18.4	18.4	46.0	
Linseed oil	18.6	18.6	18.6	18.6	46.5	
Total amount ...	37.0	73.6	73,6	37.0	129.1	
Milk kg/day.. ...	8.04	7.09	6.09	7.61	10.66	
Fat kg/day	0.428	0.415	0.342	0.358	0.559	
Fat %	5.32	5.85	5.61	4.71	5.24	
Samples	Fatty acid percentages of total fatty acids					
	3	7	6	3	6	8
Fatty acids:						
4:0	2.70	2.75	2.58	2.63	3.05	3.05
5:0	0.12	0.10	0.12	0.13	0.05	< 0.02
6:0	1.96	1.86	1.88	1.90	1.73	1.76
7:0	0.11	0.09	0.14	0.12	0.05	< 0.02
8:0	1.15	1.18	1.30	1.08	1.12	1.02
9:0	0.14	0.14	0.18	0.12	0.06	< 0.02
10:0	2.72	3.03	3.63	2.38	2.73	2.13
10:1	0.43	0.43	0.54	0.44	0.40	0.25
11:0	0.20	0.17	0.30	0.22	0.14	< 0.02
12:0	4.09	4.52	5.41	3.64	3.74	2.65
12:1	0.12	0.19	0.20	0.19	0.18	0.08

Samples	Fatty acid percentages of total fatty acids					
	3	7	6	3	6	8
13:obr	0.10	0.15	0.20	0.15	0.17	0.03
13:0	0.42	0.43	0.55	0.51	0.26	0.04
14:obr	0.15	0.37	0.18	0.32	0.21	0.09
14:0	11.05	11.31	11.99	10.17	10.95	9.87
14:1	1.42	1.35	1.63	2.63	1.95	1.01
15:obr	0.70	0.80	0.80	0.52	0.82	0.50
15:0	4.32	3.63	4.09	4.88	2.70	0.90
16:obr	0.58	0.56	0.11	0.48	0.33	0.19
16:0	48.70	42.26	40.29	48.16	38.90	28.41
16:1	3.16	3.37	3.54	4.29	4.22	2.03
17:obr	0.61	0.86	1.08	0.94	0.96	0.58
17:0	1.25	1.11	1.21	1.57	0.90	0.46
17:1	0.66	0.69	0.77	1.13	0.77	0.39
18:0	1.30	1.94	1.93	0.98	2.58	12.29
18:1	9.37	14.07	13.18	8.35	19.14	29.37
18:2	1.73	1.66	1.48	1.40	1.15	1.79
18:3	0.74	0.58	0.68	0.67	0.74	1.11

Preliminary studies on milk lipoprotein material, prepared from the buttermilk and butter serum of washed cream, have been made. The yield of lipoprotein from both the test and normal milk was about 1 % of the milk fat, in accordance with published values (King 1955), and the weight of protein remaining after lipid extraction comprised some 40 % of the lipoprotein of both test and normal milk. The nitrogen content of the protein of the lipoprotein of both milks was about 14.0 %. The amino acid composition of this minor protein of the test milk agreed almost exactly with that of normal milk, in analogy with the major milk proteins, the individual figures being similar to those reported elsewhere.

The vitamin content of 0-milk

The vitamin content of the 0-milk has been followed continuously by making determinations of the vitamin-B complex by microbiological methods. The values from the year 1963 showed that the contents of thiamine, pyridoxine, folic acid, biotin, and B_{12} were on much the same level as in normal milk. The contents of riboflavin, nicotinic acid and especially pantothenic acid were generally higher in the 0-milk than in

normal milk. The values from the later years are otherwise similar to the earlier values, but statistically significant differences between 0-milk and normal milk regarding the contents of riboflavin and nicotinic acid could not now be found (Table 4). Judging by the vitamin estimations, the biosynthesis of the vitamin-B complex brought about by the micro-organisms in the rumen seems to be rapid enough to keep up a normal content of these vitamins in the 0-milk.

TABLE 4

Vitamin content of 0-milk and normal milk during the years 1964-65 (M. Saarivirta)

		Test cows	Mixed normal milk
Thiamine-HCl	µg/100 ml	45.7 ± 1.8	43.4 ± 2.6
Riboflavine	»	306 ± 18	293 ± 32
Nicotinic acid	»	167 ± 4.9	158 ± 8.4
Pyridoxine	»	57.8 ± 5.2	53.0 ± 6.3
Folic acid (1)	»	2.91 ± 0.15	3.18 ± 0.19
Biotin	»	3.42 ± 0.31	3.13 ± 0.84
Ca-pantothenate	»	1120 ± 55	597 ± 62
B_{12}	µmg/100 ml	455 ± 46	523 ± 48
Ascorbic acid	mg/100 ml	2.78 ± 0.12	2.25 ± 0.13

(1) Values from 1963.

Porter has recently reviewed vitamin synthesis in the rumen and has concluded that the adequacy of the synthesis of some vitamins of the B group in the rumen is questionable. Our observations made with a vitamin-free feed are in many respects enlightening and show that the bacterial flora developed through adaptation syntheses effectively all the vitamins of the B group.

FLAVOR OF THE 0-MILK

The flavor compounds of the 0-milk have drawn particular attention in this laboratory. The production of milk on purified feed as free of flavor substances as possible was necessary for the comparison of the taste and aroma of this milk and normal milk. In organoleptic tests, marked differences in the flavor between 0-milk and normal milk could not be established. This is somewhat surprising because there was

reason to expect beforehand that some of the different flavor substances found in the cow's feed would be transferred to the milk *via* the organism in amounts adequate to give a flavor effect in the milk. This has been observed to be the case in practice with some strong-smelling substances which cause characteristic off-flavors in milk. For example, the onion flavor of milk when the cows are on pasture contaminated with chive (*Allium schoenoprasum*) is well known. The substances causing this off-flavor are already relatively well known (different alkyl-S_2, especially dipropyl-S_2). A «burnt» or «scorched» flavor has been reported in the milk of dairy cattle which graze on the pastures of Queensland and New Zealand infested with land cress (*Coronopus didymus*). This objectionable off-flavor is, according to the recent report of Park, produced by benzylthiocyanate, which was discovered some years ago in this laboratory, as an enzymatic splitting product of benzylmustard oil glucoside (glucotropaeolin) in the crushed seeds of garden cress, *Lepidium sativum*, and in the green parts and seeds of *L. ruderale*. It is also formed from the same glucoside in land cress. Off-flavors of other types are also transferred from the feed, for example silage of poor quality, into the milk. Thus there is no doubt that the feed consumed by the cow could have an injurious effect on the flavor of milk. It would thus seem natural that some fodder plants with a pleasant flavor would have a beneficial effect on the taste and smell of the milk. It is, however, very difficult to observe organoleptically such positive flavor effects.

The 0-milk produced on purified nutrients offers now new possibilities of elucidating the problem of the extent to which the normal flavor substances of milk are derived from the feed of the cow or are formed in the cow's organism without the large number of different and to a great degree unknown organic compounds contained in the plant material used for feeding cows. Our studies in this field can be divided into three parts. Firstly, an analysis of the flavor substances of 0-milk and of milk produced on different normal feed (good silage with pH not exceeding 4, hay of good quality, oats, beet or pasture) is being made. Secondly, attempts are being made to clarify the flavor composition of some typical fodder plants commonly used in Finland. Thirdly, experiments have been performed with lactating cows, passing into the rumen through a plastic tube pure chemical compounds of groups, whose members have been identified as flavor compounds in fodder plants. By gaschromatographic analysis of the milk the transfer of the substances to the milk could be estimated.

In this connection it is not possible to deal with the flavor problem of milk in greater detail. It may be mentioned here only that δ-lactones, probably the most important flavor substances of milk, occur in 0-milk on the average at least on the same level as in normal milk. The δ-lactones from C_6 to C_{10} are on the same, whereas the C_{12}-lactone is

on a higher level in 0-milk than in normal milk. Methods for gaschromatographic analysis and mass- spectrometric identification of δ-lactones and other flavor compounds in milk have been developed in this laboratory.

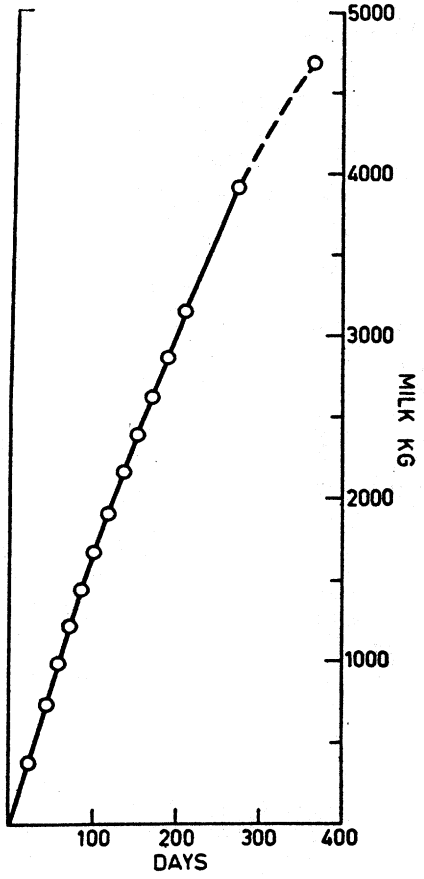


Fig. 6.—The production of milk of a cow on a urea-rich feeding described in the text.

The studies of milk production on the experimental feeding have opened new possibilities for investigations of the biosynthesis of different milk components. The studies are also of practical importance: since the vigorous biosynthesis of proteins from simple nitrogen compounds in the rumen of adapted cows has been demonstrated there are better possibilities for studies of the replacement of protein by urea. In countries with plenty of forests, part of the feed of cows can be made up of certain wood products, for instance of hemicellulose and

cellulose of low quality. The new findings may also be of value in the dry areas of the globe where milk would be of vital significance for the improvement of the nutrition of the population.

Also experiments of a practical nature are in progress. An experiment in which the cow has been given as the source of carbohydrates potatoes, sugar beet pulp, hemicellulose prepared from wood, cellulose (the so-called 0-fibre which is a waste-product of the cellulose industry), and in addition 440 g urea per day, has given excellent results This practical feeding experiment which has included a considerable amount of hemicellulose and 0-fibre has been going on at the Biochemical Research Institute since the beginning of this year when a heifer which calved at the end of February was transferred to the test feed. After calving the daily fodder dose of the cow has contained 20^{-10} kg potatoes, 3.5 kg dry sugar beet pulp, 2.3 kg hemicellulose and 2.3 kg 0-fibre and in addition 1.5 kg oat straw. The daily amount of urea has been 440-450 g, whereas the amount of true protein has been on nitrogen basis only about 20 % of the total nitrogen. About 600 g of minerals and a necessary amount of A and D vitamins have been given. On such a feed in which urea forms the main part of the nitrogen nutrition, an annual yield of about 4900 kg 4 % milk will be achieved on the basis of the production of milk so far (Fig. 6). This experiment of a practical nature in which potatoes and sugar beet pulp are included because of their low protein content shows a way to further investigations. At different regions of the world there are different plants rich in carbohydrates available, for example sugar cane, from which a part of cattle fodder can be obtained.

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SUMMARY

Experiments which have lasted for several years have shown that a considerable milk production can be achieved with cows on a protein-free feed. This has been proved decisively with feeding experiments in which purified starch, sucrose and α-cellulose have been used with urea and a small amount of ammonium salts as the sole source of nitrogen. Using 650 g urea per day during the best production period the annual milk yield has risen above 4000 kg standard milk (584 Kcal/kg milk). The investigations have shown that the composition of the milk protein has been similar on the protein-free test feed as on normal feed. The content of the water-soluble vitamins of the milk has been on the same level as well. The composition of the milk fat depends, however, on the nature and quality of the fat included in the feed. The milk flavour has been very similar to that of milk produced on normal feed.

Investigations on a protein-free feed have opened new possibilities for milk production. As proteins can be replaced by urea, which large-scale industry can produce

cheaply to an almost unlimited extent, protein-poor fodders, the utilization of which has earlier been practically impossible due to the lack of protein, can form the ration of milking cows. The feeding experiments of a practical nature which are at present in progress, have shown that in countries rich in forests a considerable part of the feed can be prepared from wood. The hemicellulose fraction can be obtained in solution with steam under elevated pressure. Sugar cane is the cheapest carbohydrate fodder in those areas where it is grown. Rations made up of different carbohydrate sources can be used in various parts of the globe.